*Environmental Health Perspectives*
*Vol. 24, pp. 133–146, 1978*

# Chemistry of PCBs and PBBs

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## Introduction

A recent book by Hutzinger et al. (*1*) reviews the chemistry of chlorinated biphenyls through the end of 1973. This wide-ranging review also includes discussions of metabolism and determination of chlorinated biphenyls.

The present report reviews areas of chlorobiphenyl and bromobiphenyl chemistry held to be pertinent to the Subcommittee's concern for possible health effects of these types of compounds. No attempt at complete coverage of these subject areas is claimed. Findings reported since 1973 are particularly stressed.

Nonmetabolic alteration of the halogenated biphenyls is considered in terms of agents most available to induce chemical change in the environment. Expected differences between chlorinated biphenyls and brominated biphenyls in their environmental chemistry are commented on.

It is possible that certain of the chlorinated biphenyl compounds will be shown to have relatively greater potential hazard for health. Therefore, occurrence and fate in the environment and metabolism and toxic effects in animals are related to chemical structure of the biphenyls.

For the general public, food is presumed to be the major source of chlorinated biphenyl compounds. The important concepts related to analytical methodology for PCB (and PBB) residues, particularly in foods, are reviewed. Alternative means of quantitation of these residues and problems in the quantitation techniques are discussed.

The pertinent aspects of chlorinated dibenzofurans, as known contaminants in commercial PCBs and as potentially significant environmental contaminants, are reviewed.

In reviewing the chemistry related to health effects of the PCBs and PBBs, it must be remembered that environmental contamination by these two commercial chemical mixtures is vastly different in magnitude. The former have been steadily released into the environment, in many countries, presumably over decades, and are now found to be a pervasive, world-wide contaminant. The number of chlorinated biphenyls reaching the environment probably number nearly 100 different compounds. The PBBs, encompassing a small number of chemical structures to begin with, are of concern due to a single, fairly recent contamination incident, apparently limited to the State of Michigan.

## Chemistry of Chlorinated Biphenyls

### Synthesis and Analysis of Commercial Mixtures

Commerical preparation of mixtures of chlorinated biphenyls by reaction of biphenyl with chlorine has been described by Hubbard (*2*). A more recent discussion of this subject, including the newer preparation designated as Aroclor 1016, is now available (*3*). Analysis of the chlorinated biphenyl content of various American commercial PCB mixtures (Aroclors) has been reviewed by Hutzinger et al. (*1*). An estimated 40 to 60 different chlorinated biphenyl compounds are present in each of the higher chlorinated commercial mixtures. (There are 209 possible compounds obtainable by substituting chlorine for hydrogen on from one to ten different positions on the biphenyl ring system). PCB commercial mixtures produced in the U. S.

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and elsewhere have been shown to contain classes of compounds other than the chlorinated biphenyls: chlorinated naphthalenes and chlorinated dibenzofurans (Cl-DBFs) (*4–7*). The possibility that naphthalene and dibenzofuran contaminate the technical biphenyl feedstock used in preparation of the commercial PCB mixtures has not been excluded.

Available toxicity information indicates that, of the identified types of contaminants in commercial PCB mixtures, Cl-DBFs pose the greatest potential hazard. As a result, the Cl-DBFs have become the focus of studies on contaminants in these mixtures. No report has appeared of an attempted complete content analysis of the trace impurities in a commercial PCB mixture.

## Nonmetabolic Alteration of Chlorinated Biphenyls

Chlorinated biphenyls, as is typical of aryl chlorides generally, are quite stable to chemical alteration. Consideration of possible nonmetabolic alteration routes for these compounds in the environment suggests air oxidation, aqueous hydrolysis, and photoalteration in sunlight as potential reactions to be investigated. (Thermal decomposition would be of concern in connection with incineration conditions meant to destroy waste chlorinated biphenyls.) Study of these reactions under the complex and variable sets of conditions existing in the environment is difficult. Chemical reactivity of chlorinated biphenyls has therefore been studied almost entirely under the more controlled conditions of the laboratory.

***Oxidation.*** PCBs are fairly stable to oxidation under moderate conditions. PCBs are stable under conditions which easily oxidize DDE and can be separated from DDE by oxidizing DDE to the more polar dichlorobenzophenone prior to column chromatographic separation (*8*). To effect the oxidation of DDE, the PCB–DDE solution is refluxed in 2.5% chromic acid–acetic acid on a steam bath. Some of the lower chlorinated biphenyls, however, are not recovered after the oxidation. It is reported (*9*) that mono-, di-, and trichlorobiphenyls are oxidized in 7.5% chromic acid–acetic acid to their respective (chloro-)benzoic acids. With vigorous oxidizing conditions in the environment, some PCB oxidation, especially of the lower chlorinated biphenyls, may occur. It is difficult, however, to assess the total extent of possible environmental oxidation of PCBs.

***Hydrolysis and Alcoholysis.*** PCBs are fairly stable to hydrolysis under moderate conditions. When refluxed with 2% KOH in ethanol, PCBs are stable (*8, 10*). A halogen on a chlorobiphenyl molecule is not easily displaced in a nucleophilic substitution reaction. Under vigorous conditions, the 4- and the 4,4′-positions of decachlorobiphenyl are found to be the most susceptible to chlorine displacement by hydroxide and methoxide ions. Decachlorobiphenyl can be hydrolyzed to octachloro-4,4′-biphenylol when treated with aqueous alkali at high temperatures in an autoclave (*11, 12*). Heating 2,5-dichlorobiphenyl with sodium methoxide produces 2-chloro-5-biphenylol (*13*). Decachlorobiphenyl, when treated with sodium methoxide in pyridine, yields the 4-methoxy- and 4,4′-dimethoxychlorobiphenyl (*14*). It is probable that the nonmetabolic hydrolysis or alcoholysis of PCBs in the environment is limited.

***Photochemistry.*** Exact environmental conditions for the photochemistry of environmental contaminants, including PCBs, are sometimes difficult to simulate in the laboratory. Limited understanding exists concerning the parallel of laboratory photoreactions and reaction rates under nonenvironmental conditions and possible environmental photolyses. The exact environmental conditions under which residues may photoreact can be diverse and difficult to evaluate. Unknown or discounted factors in the environment, such as possible sensitizers or quenchers, may be important. The frequency (energy) and intensity of a laboratory photolysis light may not be comparable with environmental conditions. 3000 Å probably represents the practical lower limit of the ultraviolet portion of sunlight (*15*). The residue must be exposed to sufficient light to react appreciably. It is necessary to consider the physical state of the residue exposed to light in the environment. The residue may exist as a solid or liquid (e.g., a film), a solution, or a vapor. In photolyzing solutions the solvent can have an important effect. Photoreactions which proceed in one solvent may not proceed or may proceed at a different rate in another solvent. A muddy river containing a residue may yield different results compared to a laboratory photolysis in hexane. Laboratory photolyses under a variety of conditions which simulate most, if not all, of the environmental conditions of photolysis could be extremely significant and relevant. In the absence of these, we must rely on the questionable extrapolation of laboratory photolyses under nonenvironmental conditions.

The photochemistry of PCBs has been described by Hutzinger et al. (*1, 16*). In contrast to oxidation and hydrolysis, PCBs are fairly easily photoreacted under certain laboratory conditions. PCB photochemistry has been studied in various solutions, as a thin film, and as a vapor. In hydrocarbon solvents,

progressive reductive dechlorination of the PCB is the predominant photochemical reaction (*1*). Irradiation of solutions of chlorobiphenyls (*16*) and trapped gas liquid chromatographic (GLC) effluents (*17*) have shown that PCB with higher chlorine content dechlorinate more readily. When 0.1% hexane solutions of 2,2′,5,5′-tetrachlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl were each irradiated with 3100 Å light in a Rayonet reactor (*16*), after 24 hr of irradiation, 33% of the 2,2′,5,5′-tetrachlorobiphenyl was unreacted, while < 1% of the 2,2′,4,4′,5,5′-hexachlorobiphenyl remained. In dechlorinating PCBs by photoexcitation, *ortho* chlorines cleave preferentially (*18*). With PCBs containing only *meta* and *para* chlorines, *meta* chlorines are lost preferentially. The rate of dechlorination is faster in alcohol solvents, such as methanol (*19*) than in hydrocarbon solvents. Irradiation of 2,2′,4,4′,5,5′-hexachlorobiphenyl in methanol yields, in addition to reductive dechlorination, ring methoxylation (*18*). Photochemical studies of PCBs in aqueous solutions may offer a significant relevance to environmental PCB photochemistry. A 0.4% Aroclor 1254 suspension in water–dioxane (7:3) in the presence of sodium bicarbonate with air bubbling through the mixture was irradiated with 3100 Å light. Two thin-layer chromatographic fractions of the Aroclor 1254 after irradiation, corresponding to the "addition of water to chlorobiphenyls" and a "carboxy" fraction, were found (*16*). Presumably, PCBs formed by reductive dechlorination were also produced. Black light irradiation of Aroclor 1254 as a thin layer film in the presence of water yields a "carboxy" fraction and a fraction whose mass spectrum indicates hydroxychlorobiphenyls (*16*). Sunlight irradiation of certain chlorobiphenyls as a thin film without the presence of water gives dechlorinated products (*1*). Chlorinated terphenyls and quaterphenyls are also produced by the black light and sunlight irradiation of certain chlorobiphenyls as a thin film (*1*). Vapor phase photolysis of PCBs may be extremely relevant, especially for the more volatile components of Aroclors with low chlorine content. Sunlamp irradiation of the vapor phase of a refluxing suspension of 2,2′,5,5′-tetrachlorobiphenyl and water yields "carboxy" products (*16*).

These laboratory photoreactions represent chemical conversions which may proceed in the environment. The photoreaction rates under diverse environmental conditions and the extent of PCB degradation or reaction in the environment are extremely difficult to assess.

The reported low-yield conversion of certain chlorinated biphenyls to Cl-DBF is discussed further below.

## Chemical Structure Related to Occurrence, Fate, and Effects of Chlorinated Biphenyls

The physical and chemical properties of chlorinated biphenyl compounds and commercial mixtures of PCBs vary greatly, depending on the degree and position of chlorine substitution on the biphenyl ring system. Of particular importance to their environmental occurrence and fate are the properties of volatility, water solubility, bioaccumulation, biodegradability, and photostability. Volatility, water solubility and bioaccumulation are of more importance as mechanisms of introduction into and transport within the environment. Biodegradation and photodegradation are more important as mechanisms of removal.

There are data (*3*) which indicate that the higher chlorinated biphenyls are less volatile and less water-soluble than the lower chlorinated biphenyls. These two factors would tend to enhance the ratio of lower to higher chlorinated biphenyls in the environment. There are no clear data to assess the involvement of chlorine position in volatility and water solubility, but this is likely to be of less importance. Therefore, the lower chlorinated biphenyls are more likely to volatilize into and be selectively transported in the aqueous environment.

The relationship between structure and bioaccumulation factors for PCB isomers are only beginning to be assessed (*20, 21*) with the availability of purified specific isomers for study. However, it appears (*22, 23*) that the peak patterns from gas chromatography found in environmental samples representing the biosphere most closely resemble the higher chlorinated commercial mixture, Aroclor 1254. This suggests a selective accumulation of the more highly chlorinated components of the commercial mixtures in biological material. This also indicates that the higher chlorinated biphenyls are able to find their way into the environment in spite of their poorer volatility and water solubility. Pharmacokinetic studies (*24*) with selected radiolabeled chlorinated biphenyl compounds have confirmed the trend toward increasing biological half-life with increasing chlorine number. However, these findings may be the result of both higher accumulation rates and lower elimination rates for the higher chlorinated biphenyls. It may be of interest to note that fish from lower and intermediate levels of the food web have been found (*22*) to contain lower amounts of hexachloro and higher amounts of tetrachloro and pentachloro compounds than higher trophic level white and silky sharks and aquatic birds. This suggests that selectivity in bioaccumulation is also a function of biospecies.

Again, the effects of varying chlorine position are

less clearly understood, and the best assessment of this comes from study of two or more members of an isomeric series of the chlorinated biphenyls. Recent work with five symmetrical hexachlorobiphenyl isomers in chicks (*25, 26*) and in mice (*27*) has demonstrated that separate and distinct differences in isomer toxicity are possible and that the differences are related to chemical structure via effects of varying chlorine substitution on compound lipophilicity and metabolism. The hexachloro isomers studied were 3,4,5,3′,4′,5′-; 2,4,6,2′,4′,6′-; 2,3,-4,2′,3′,4′-; 2,4,5,2′,4′,5′-; 2,3,6,2′3′,6′- and 2,3,-5,2′,3′,5′-chloro compounds. Those hexachloro isomers with 4,4′-substitution appear to be more rapidly accumulated and may be more slowly metabolized, i.e., these isomers showed greater accumulation in adipose tissue and increased activity in the liver and overall greater toxicity. These differences are believed to be associated with differences in molecular polarizability and to be measurable by spectroscopic and chromatographic techniques.

Other workers (*28, 29*) have compared the toxicity of two pentachlorobiphenyls in laying chicks. The isomer with 4,4′-substitution (2,4,5,3′,4′-pentachloro) showed higher average embryonic mortality and teratogenicity in unhatched eggs but lower decreased fertility in the chick than the 2,3,6,2′,3′-pentachloro compound, an isomer not chlorinated in the 4-position. Although it is difficult to assess the role of metabolism here, the data would suggest a more rapid accumulation of the 2,4,5,3′,4′-isomer in the egg. Other workers (*30*) have already observed a correlation between PCB content of eggs and embryo mortality and teratogenicity.

Various other workers (*31–33*) studying a range of chlorinated biphenyls have generally observed the importance of degree of chlorination as well as position of chlorine substitution (especially 4,4′-substitution) in overall biological effectiveness. Although some of the compounds studied are unrealistic as components of commercial PCB mixtures, they have served as models to demonstrate the importance of chlorine number and position. Nevertheless, of 50 possible components of Aroclor 1254 identified by Sissons and Welti (*34*), as many as 19 (38%) could have 4,4′-substitution. It is of interest to note here that one of the possible persistent isomers found in Yusho patient tissue has been tentatively identified as the 2,3,4,5,3′,4,′-hexachlorobiphenyl (*35*). A structurally similar isomer (2,3,4,5,3′,4′,5′-heptachlorobiphenyl) has been prepared (*36*), and its toxicity will be determined especially in relation to the highly toxic 3,4,5,3′,4′,5′-hexa isomer previously tested.

It has been only recently that hydroxylated metabolites of PCBs have been isolated and identified in environmental samples (*37*). The study of the metabolism of the commercial mixtures themselves (*38*) has received little attention for obvious reasons. Recently, the metabolism of purified chlorinated biphenyls, some radiolabeled for quantitation purposes, has been studied in a number of biological systems. Although there are a large number of publications in the literature on this subject, this report will concern itself with those studies which seem to support generalities in terms of the effects of varying chlorine number and position on metabolism with particular reference to mammalian systems and to realistic components of Aroclors.

The distribution and excretion of a series of four radiolabeled ($^{14}C$) chlorobiphenyls which have degrees of chlorination similar to and are themselves constituents of Aroclors 1221, 1242, 1254, and 1260 have been studied in the male rat (*39*). These studies have clearly shown an increasing biological half-life with increasing chlorine number for the series (4-; 4,4′-; 2,4,5,2′,5′-; and 2,4,5,2′,4′,5′-) which is believed to be related to the rate of metabolism and the ease of formation of the arene oxide intermediate. These compounds were also dosed at realistic exposure levels (0.06 to 6.0 mg/kg) which showed that little if any of the chlorobiphenyl is excreted in unchanged form. Most other metabolic studies (*40*) with selected chlorobiphenyls have dealt with much higher dose levels (generally resulting in excretion of much unchanged biphenyl) and unlabeled compounds which are of little quantitative value in assessing the effects of chlorine degree and position.

There has been no similar study with selected chlorinated biphenyls within an isomeric series to assess the effects of chlorine position on metabolism with the possible exception of an incomplete study (*41*) on the identification of metabolites from the excreta of chicks (and mice) fed symmetrical hexachlorobiphenyl isomers. It is essential that this be done with symmetrical isomers (in order to simplify the problem of interpretation), preferably in two or more isomeric series (tetra- and hexachlorinated).

The various metabolism studies have generally shown the occurrence of polar hydroxylated compounds as major components of the metabolite mixture. As the degree of chlorination increases, hydroxylation can be concurrent or concomitant with dechlorination (*40*). There is also evidence for the formation of methyl ethers (*42*) and methylchlorobiphenyls (*41*) as metabolites.

There is an increasing body of evidence to support the arene oxide intermediate in hydroxylation

(*43*), especially in cases where vicinal (1,2 or adjacent) unsubstituted carbons are found in the molecule (*44*). The corresponding dihydrodiol and/or catechol generally occur along with the phenolic metabolites.

Although it has not been studied in mammalian systems, there is at least one report (*45*) that studies of individual chlorinated biphenyls do not accurately predict the rates of metabolism of the same compound in simple mixtures. Therefore, the overall problem of metabolic degradation of the commercial mixtures may be complicated by the possibility that certain PCBs are potent enzyme inducers but poor substrates and vice versa, with various degrees in between. This is further complicated by the fact that certain chlorinated biphenyls occur in optically active forms and only one enantiomer may be biologically active and undergo enzyme interactions. The existence of nine of the major, and ten of the minor, constituents of Aroclors 1242, 1254, and 1260 in optically active forms has been predicted (*46*).

## Determination of PCB Residues

The determination of PCB residues has been reviewed in detail by the WHO Task Group report on Environmental Health Criteria for Polychlorinated Biphenyls and Terphenyls (*47*) and by Hutzinger et al. (*1*).

PCBs are lipophilic, quite similar in this respect to DDE, the metabolite of DDT. Analyses for PCB residues follow procedures the same as or similar to those used for multiple residues of organochlorine pesticides (*40, 47–50*). The individual steps that follow sampling are extraction of residues from the sample, isolation of residues from coextractives (cleanup), separation of PCBs from interfering chlorinated hydrocarbon pesticides, quantitation, and confirmation of identity. In general, analytical methods capable of completely extracting residues of organochlorine pesticides from sample substrates and of quantitatively recovering the pesticides through subsequent cleanup procedures are also capable of achieving quantitative analysis for PCBs. The lipophilicity of chlorobiphenyls, which increases with increasing chlorine content, may influence their recovery through some analytical methods (*51*). For example, recoveries through the frequently used cleanup step, partitioning of PCB from a petroleum ether or hexane solution of fat or oil to acetonitrile, exceed 95% for Aroclor 1242 but drop to 75–80% for Aroclor 1260 (*50*). Overall ability of typical analytical methodology to recover PCBs added *in vivo* to food samples is shown by two interlaboratory studies conducted within the Food and Drug Administration (FDA) and one study conducted for the Association of Official Analytical Chemists (AOAC) (*52–55*). Using methodology described in the FDA Pesticide Analytical Manual (*48*) and in the Book of Official Methods of the Association of Official Analytical Chemists (*49*), average recoveries and coefficients of variation for determination of Aroclors added as unknowns to different foods were: Aroclor 1254, fish, 74 ± 9%; Aroclor 1254, infant chicken, 89 ± 22%; Aroclor 1242, chicken fat, 101 ± 13%; Aroclor 1248, chicken fat, 96 ± 9%; Aroclor 1254, fish, 75 ± 14%; Aroclor 1260, fish, 75 ± 15%. The three studies involving fish required separation of the PCBs from the DDT group before quantitation.

Gas chromatography with electron-capture detection is the most widely used procedure for determination of PCB residues. Gas chromatographic columns with methyl silicone liquid phases are widely used under conditions that typically separate the Aroclors and PCB residues into about 15 peaks (*56*). The 10% DC-200 (or OV-101) column described in the FDA Pesticide Analytical Manual (*48*) separates Aroclor 1254 into 14 peaks (*57*). These analytical columns do not give a highly defined representation of the Aroclor or PCB residue; several of the peaks in Aroclors result from mixtures of more than one chlorobiphenyl (*34, 58–60*). A column prepared from purified Apiezon L has been shown to separate a 54% chlorine PCB mixture into over 40 peaks (*61*). Separation of the PCB residue into three fractions by chromatography on charcoal prior to examination on the Apiezon L column has made it possible to characterize and quantitate nearly 60 technical PCB components. This method provides a way to get much needed, more detailed information on the composition of PCB residues and might be used in special studies. The increased time and complexity of this approach would probably limit its application in regular monitoring analysis.

The need to confirm residue identity and the procedures available are similar for PCBs and pesticides. The multipeak gas chromatographic pattern of a PCB residue may be very similar to that of a commercial Aroclor or, as in most biological samples, the residue peak pattern may be changed to varying degrees from that of a given Aroclor. The use of column chromatographic or chemical reaction procedures to separate PCBs from organochlorine pesticides increases the certainty of the gas chromatographic identification. Procedures readily available to the residue laboratory for confirming the identity of PCBs include: halogen specific gas chromatographic detectors (*50*), stability of the residue peak pattern after refluxing the

extract with alcoholic alkali solution (*10*), perchlorination of the residue to the decachlorobiphenyl derivative (*62, 63*), and thin-layer chromatography (*64, 65*). Mass spectrometry is not readily available to many laboratories conducting analyses for PCBs nor would the expense justify regular use in monitoring programs. However, the use of mass spectrometry is encouraged for residues and samples which are unusual or significant and for the occasional examination of a so-called routine sample.

Two especially critical considerations are associated with the determination of PCB residues: (1) their separation from potentially interfering organochlorine pesticides, particularly DDT, TDE, and DDE or the multicomponent chlordane or toxaphene, and (2) quantitative measurement of the multicomponent PCB residue which, in biological organisms, is usually changed in relative amounts of chlorobiphenyl components from commercial Aroclors or which may result from mixture of PCBs from different sources. To achieve reliable residue results it is essential that the analyst correctly make critical judgements and interpretations in dealing with mixed residues of PCBs and organochlorine pesticides and in quantitation of the multicomponent PCB residue. There is no substitute for analyst experience in this analysis.

PCBs are separated from certain pesticides in the usual cleanup procedures, e.g., adsorption chromatography on Florisil or alumina or by gel permeation chromatography (*48, 66, 67*). When DDT, TDE, DDE, chlordane, or toxaphene are present, ancillary procedures designed to separate these chemicals from PCBs must be used. The DDT analogs, particularly DDE, are the pesticide residues most frequently encountered in samples and DDE is the most difficult to separate from the PCBs. The effective electron capture gas chromatographic response for DDE is 20-30 times that for an equal weight of Aroclor 1254, and the response for DDT is only slightly less than that for DDE (*48*). Without proper treatment these pesticides can readily interfere in the determination of PCBs. Some procedures utilizing column chromatography or alumina or Florisil separate DDT and TDE and intentionally collect DDE and PCBs in the same fraction (*67, 68*), which is analyzed. In these cases the analyst must exclude the DDE region of the gas chromatogram from the quantitative measurement of PCBs. Column chromatography on charcoal also has been proposed for separation of PCBs from DDE, DDT, and other organochlorine pesticides (*62*). Column chromatography on silicic acid is probably the most widely used procedure for separating PCBs from DDT and analogs (*48, 57*). The technique is fairly lengthy and difficult to reproduce, requiring empirical standardization in each laboratory (*55, 69, 70*). The separation of DDE is probably not 100% effective, but under ideal conditions practically all DDE can be separated; the usual inconsistency is for a portion of the DDE to be eluted from the column with the PCBs, requiring allowances to be made in PCB quantitation. The lower chlorinated PCBs present the greatest difficulty in separation from the DDT group by column chromatographic procedures.

DDE can also be separated from PCBs by oxidation to the dichlorobenzophenone, followed by a chromatographic separation of this more polar derivative from PCBs. DDT and TDE may be dehydrochlorinated to their respective olefins and similarly separated along with DDE (*8, 71, 72*). This technique has not received as much application as the chromatographic procedures, probably because pesticides and lower chlorinated biphenyls are destroyed or changed, preventing their determination. A variation of this approach used sodium dichromate plus a minute amount of sulfuric acid, rather than dehydrochlorination followed by oxidation with chromium trioxide in acetic acid. The dichromate reagent is reported to convert DDE quantitatively to the dichlorobenzophenone without affecting DDT, TDE, or any of the chlorobiphenyls (*61*).

Quantitation of PCB residues is done in most laboratories by comparison of measurements made on the multicomponent electron-capture gas chromatograms of the residue and a known quantity of reference material. The PCB residue in biological samples, as mentioned earlier, is very likely to be changed in the relative amounts of chlorobiphenyl components from any one Aroclor or the residue may be a mixture of PCBs from different sources (*48, 61, 73*). The response of the electron capture detector varies with the number and location of chlorine atoms in the biphenyl molecule (*74, 75*). With the electron capture system described in the FDA Pesticide Analytical Manual (*48*), the response/unit weight increases about 6-fold from Aroclor 1242 to Aroclor 1260 (*50*). The halogen-specific microcoulometric and electrolytic conductivity detectors respond proportionally to the weight of chlorine present and in theory could provide a more accurate measurement of a PCB residue that is not exactly the same composition as the reference (*50*). However, lower sensitivity, difficulty in maintaining optimum performance, and limited availability in residue laboratories has limited the use of these detectors. Unless the PCB residue and reference Aroclors are exactly the same in chlorobiphenyl composition, the gas chromatographic determination cannot be considered accu-

rate; the greater the difference between residue and reference composition, the greater the deviation between determined and true residue level (*50, 76*). The most frequently used approaches to quantitation have selected for the reference the Aroclor with the most similar gas chromatographic pattern to the residue and: (1) compared the response of a single peak in the residue with the response of the counterpart peak in the Aroclor reference; or (2) compared the total response for several peaks from the residue to the total response for the counterpart peaks in the reference; or (3) compared the total response for all peaks in the residue with the total response for all peaks in the reference; or (4) in a greater effort to duplicate the residue peak pattern, prepared a reference made up of one or more Aroclors to simulate the gas chromatographic pattern of the residue, and compared the total response for all peaks of the residue to the total response for all peaks in the reference (*55, 76*). Response has been measured in terms of both peak height and area (*55*). The latter approach, which utilizes an Aroclor or a mixture of Aroclors to duplicate the residue peak pattern, is recommended by the AOAC for quantitation of PCB residues in certain foods (*49*). This is a currently accepted and practical way to quantitate PCB residues. Perchlorination of PCBs to decachlorobiphenyl has been suggested as a means to improve precision and consistency in PCB determination (*61, 62*). This approach will not improve the accuracy of quantitation, however, because proportions of individual chlorobiphenyls in the PCB residue remain unknown; equal weights of individual chlorobiphenyls of different chlorine content result in different weights of decachlorobiphenyl. Contamination found in antimony pentachloride, the perchlorination reagent, also detracts from this procedure for quantitation purposes (*77*). An approach to quantitation which appears to have practical merit as well as offering improved accuracy in quantitation has been advanced by Webb and McCall (*60*). The PCB residue is quantitated peak by peak in comparison to reference Aroclors which have been characterized as to the number of chlorines and the fraction of total Aroclor weight represented by each electron capture peak after separation on a widely used (methylsilicone liquid phase) gas-chromatographic column. The availability of carefully characterized Aroclors and evaluation in practice are required to fully evaluate the merits of this procedure.

Precision in the interlaboratory determination of PCBs is slightly less than with the common organochlorine pesticides. In several interlaboratory studies involving biological samples and paperboard containing either added Aroclors or actual residues of PCB, the coefficients of variation are about ± 20%. The results of studies conducted by the AOAC and the FDA involving samples containing added Aroclors have been mentioned above (*52, 54, 55, 78*); the recoveries of added Aroclors ranged from 74 to 101% and coefficients of variation from 9 to 15%. The levels of Aroclors added in these studies ranged from about 2 to 8 ppm with the exception of 0.2 ppm Aroclor 1254 added to chicken infant food. With samples containing actual residues, nine laboratories in the AOAC study reported 9.2 ppm ± 8% for a residue in chicken fat and 4.5 ppm ± 20% for a residue in Lake Michigan chubs. The residue in the chubs was determined after separation of DDE, DDT, and TDE by column chromatography on silicic acid. In a study by eight laboratories in cooperation with the International Council for the Exploration of the Sea, PCB residues determined in a fish oil averaged 1.97 ppm ± 47%. A much better coefficient of variation, about ± 11%, was obtained when the same fish oil was fortified with an additional 10 ppm PCB (*79*). In an AOAC study of the method for PCB in paperboard, 11 laboratories analyzed a paperboard sample manufactured to contain Aroclor 1242 and reported 5.6 ppm ± 16% (*80*). The inter- or intralaboratory precision of the determination of PCB in any sample type is improved by use of the same analytical procedures, especially in quantitation of the residue.

The lower limit of quantitation for PCB residues will vary among laboratories, depending upon the objectives of their analyses and upon the particular details of the analytical methods, especially the sensitivity of gas chromatographic detection and the sample size. The quantitation limit achieved will be higher than for organochlorine pesticides because of lower effective detector response to the technical PCB mixtures; 20-30 times more Aroclor 1254 or 30-50 times more Aroclor 1242 than DDE is required for the major peaks to produce the same peak height as DDE in electron capture gas chromatography (*48*). Other factors which may restrict the attainment of low limits of quantitation and affect analytical reliability are interfering residues in the sample and laboratory contamination. Fish from some locations contain residues of organochlorine pesticides and possibly other contaminants at levels that would cause increasing difficulty as the PCB residue decreases below about one ppm. Contamination of laboratory equipment, reagents, and samples with PCB from containers, previous samples, control runs, and unknown sources, must be carefully guarded against in analyses where a low level of quantitation is necessary, for example with human blood or certain environmental samples (*8,*

*81, 82*). Perchlorination of the PCB residue to decachlorobiphenyl offers the possibility of about 25 fold increase in sensitivity in the gas chromatographic determination (*63*). However, contaminants present in antimony pentachloride severely restrict or prohibit application of this procedure to determination of low levels of PCBs (*77*). Analytical methods used by the FDA for PCB residues also recover chlorinated naphthalenes (*83*). If present alone, chlorinated naphthalenes would be recognized by the analyst. However, their presence in admixture with PCBs would probably go unrecognized unless amounts were greater than PCBs or the residue was examined by mass spectrometry. Chlorinated naphthalenes are oxidized by procedures used to differentiate DDE in the presence of PCBs and could be removed from interfering in the PCB determination (*84*).

From examination of procedures used to analyze for chlorinated dibenzofurans in commercial PCB formulations and from analytical studies with chlorinated dibenzodioxins it can be inferred that some chlorinated dibenzofurans may be recovered along with PCBs through analytical methods using Florisil and column chromatography (*4, 6, 7, 85*). The chlorinated dibenzofurans have gas chromatographic properties similar to those of the PCBs (*7*). It is very doubtful, however, that a relatively small amount of chlorinated dibenzofuran in the presence of PCBs would be recognized in the usual analysis for PCBs.

Little study has been made of residue analytical methodology for chlorobiphenylols (hydroxylated chlorinated biphenyls). Procedures used for their determination differ substantially from those used for PCBs (*86, 87*). It is not likely that chlorobiphenylols would be recovered through column chromatographic and separation procedures used for PCBs.

In the discussion of PCB residues in foods given in the report of the Human Exposure Group, a large share of the information comes from the FDA's surveillance programs. The analytical methodology used in the analyses is described in the FDA Pesticide Analytical Manual, Vol. I (*48*), and in Official Methods of Analysis of the AOAC (*49*). Quantitation of PCB residues is by gas chromatography with electron capture or halogen specific electrochemical detectors. The total response (area or peak height) for the PCB residue is compared to the total response for the Aroclor reference (or mixture of Aroclors) having the most similar gas chromatographic pattern. Aroclor reference materials are each from a single master lot. Results are reported as parts per million of the Aroclor(s) used for the quantitation reference. With certain exceptions the limit of quantitation for PCBs (based on the electron-capture detector response to Aroclor 1254) is about 0.2 ppm for individual foods and about 0.05 ppm for total diet composites. Fish from certain fresh water locations are the food most frequently and consistently found to contain PCB residues. The residues in fish are most often similar in gas chromatographic peak pattern to Aroclor 1254 but usually with higher concentrations of the late eluting (higher chlorination) chlorobiphenyl components.

## Chlorinated Dibenzofurans

In considering the potential hazard to humans of the commercial chemical mixtures of chlorinated biphenyls called PCBs, one must consider which, if any, identified trace contaminant in these complex mixtures might contribute significantly to the overall hazard potential of the mixture. Although there is evidence for the presence of chlorinated naphthalenes and possibly chlorinated terphenyls in PCBs, the chlorinated dibenzofuran (Cl-DBF) contaminants are regarded as a greater potential danger for several reasons. In using a chick bioassay to monitor fractionation of commercial PCB mixtures, it was found (*4*) that the fraction most toxic to chicks, and far more toxic than the other fractions, contained Cl-DBFs and chlorinated naphthalenes. What limited toxicological information there is available on these halogenated naphthalenes, terphenyls, and dibenzofurans suggests that only the Cl-DBFs may be more toxic than chlorinated biphenyls by orders of magnitude (*88, 89*). The extremely high toxicity of some of the chlorinated dibenzo-*p*-dioxins (*90, 91*), a class of organic chemicals very similar in structure to the Cl-DBFs, also suggests the latter are likely to be highly toxic. Knowledge of the chemistry and toxicity of various chlorinated dibenzo-*p*-dioxin structures and the relationship between toxicity and structure, although recently developed, is more complete than for Cl-DBFs. Therefore, findings in the chlorodioxin field provide indicators to assess the difficulties likely to be encountered in the study of Cl-DBFs.

Chlorinated dibenzofuran contaminants have been reported and confirmed in PCB mixtures manufactured in Germany and France (*4, 7*), in Japan (*5, 92*), and in the United States (Aroclors) (*6, 7*). An additional claim that a Cl-DBF contaminated an Aroclor was not sufficiently supported by the evidence presented (*93*). In the PCBs of U. S. manufacture, the range of Cl-DBFs found was from dichloro through hexachloro, and sufficient evidence was obtained to show that 2,3,7,8-tetrachloro- and 2,3,4,7,8-pentachlorodibenzofuran

are present in Aroclors (*94*). That the Cl-DBFs reported were actual contaminants and not artifacts resulting from the experimental procedures used, was considered in two instances (*6, 92*).

Proper quantitation of the individual Cl-DBF contaminants is very difficult. It requires full structural identification of the Cl-DBF contaminant, availability of the contaminant as a highly purified reference standard for quantitation purposes and evaluation of the procedure used to concentrate and separate the Cl-DBFs (e.g., from chlorobiphenyls) to determine the capability of the procedure to recover the specific Cl-DBFs in the PCB mixture. Reported attempts to quantitate the Cl-DBFs in the PCBs suggest contamination levels in the low parts per million range for total chlorinated dibenzofurans (*7, 92*). Many of the reported values probably can be taken as minimal in the absence of recovery data.

Although the source of the Cl-DBF contaminants in commercial PCB mixtures has not been determined, several possibilities may be considered. The simplest explanation would be the likely presence of dibenzofuran (parent compound) in the technical grade biphenyl subjected to the chlorination process. From a consideration of the procedures used in the commercial synthesis of PCBs (*2*), various chlorinated biphenyls, if substituted in the *ortho* and *ortho*-prime positions with hydroxy groups and/or chlorine atoms, might ring close to form the furanoid ring by dehydration or dehydrochlorination.

It is important to recall that Cl-DBFs have been reported as contaminants also in other chemicals of commercial importance. Samples of pentachlorophenol (*95*) as well as lower chlorinated phenols (*96*), and hexachlorobenzene (*97*), have been found to contain a range of Cl-DBFs. The ppm level of Cl-DBFs in certain pentachlorophenols examined by Schwetz and co-workers (*95*) was considerably higher than the highest level thus far reported in commercial PCB mixtures; the chlorine level in the reported dibenzofuran contaminants of pentachlorophenol ranged from hexa to octa.

It is now known that "PCB residues" obtained from environmental samples usually do not simply represent an easily identified original commercial PCB mixture. For example, there is evidence from the study of electron-capture gas chromatograms that certain chlorinated biphenyl compounds are preferentially lost or concentrated in passing through environmental media. This has led to recent studies of the toxicity, metabolism and physiological effects of specific, individual chlorinated biphenyl compounds, synthesized by routes intended to yield a single compound of known structure. In addition to permitting the development of structure-activity correlations, these studies presumably were intended to avoid complications in interpreting results from the testing of the commercial chlorinated biphenyl mixtures (*98*), now known to contain toxic Cl-DBFs as contaminants. Interpretation of results from the testing of individual chlorinated biphenyl compounds, however, may not be entirely straightforward. In the Ullmann coupling reaction of 2,4,5-trichloroiodobenzene, it has been reported (*99*) that the highly toxic 2,3,7,8-tetrachlorodibenzofuran is formed in 3% yield in addition to the expected 2,2′,4,4′,5,5′-hexachlorobiphenyl product. This hexachlorobiphenyl is a significant constituent of PCB mixtures (*34, 61, 100*), and has been singled out for biological studies by several workers (*32, 98, 101*). Careful purification and, if necessary, chemical analysis of symmetrical chlorobiphenyls prepared by the Ullmann coupling reaction (for dibenzofuran content) is suggested as a prerequisite to biological testing of the biphenyl.

There are two other aspects of the chemical relationship between chlorinated biphenyls and Cl-DBFs that need to be mentioned. Both deal with possible environmental alteration. Evidence has been obtained for photochemical conversion of certain chlorinated biphenyls to Cl-DBFs in very low yields (*102*). Although the scope of this study was limited, formation of Cl-DBF was shown to occur under conditions of sunlight irradiation and in the laboratory using sunlight-simulating conditions. Two different *ortho*-chlorinated biphenyls (the 2,5-dichloro and 2,2′,5,5′-tetrachloro) were claimed to produce approximately 0.2% steady-state yields of a monochloro DBF. The limited reports regarding the photochemical decomposition of Cl-DBFs (*102, 103*) indicate relatively rapid destruction of the compounds may take place in the environment. Since reductive dechlorination is a major route of photochemical alteration of Cl-DBFs, the possibility of dechlorination of highly chlorinated DBFs to more toxic DBFs of lower chlorine content must be considered. The extent to which specific chlorinated biphenyls, found as major constituents of commercial PCB mixtures, can be photochemically converted to Cl-DBFs can only be determined by further experimental studies.

Analysis of the Yusho oil (the rice oil contaminated by PCB-containing heat exchange fluid implicated in the Japanese Yusho poisoning incident) by Nagayama et al. (*92*), for Cl-DBF content, led to a value of 5 ppm Cl-DBFs. This value is about 300 times the Cl-DBF level expected in the Yusho oil if one simply assumes contamination of the oil by Cl-DBFs to be proportional to the Cl-DBF level found in unused Kanechlor 400 (the Japanese PCB mixture claimed to have been used as the heat ex-

change fluid) and to the level of PCB in the Yusho oil. This led Kuratsune et al. (*35*) to suggest that Cl-DBF levels increased in the heat exchange fluid through use. Going a step further, these questions may be raised. Is Cl-DBF concentration increased, generally, in PCB-containing heat exchange fluids through use? Does such increase occur in PCB-containing transformer fluids or electrical capacitors through use?

A critical review is needed of the published data upon which Kuratsune's suggestion is based (*92*), particularly since the results from a recent analysis of a portion of Yusho oil (*104*) raises questions about the concentration of chlorinated biphenyls in the Yusho oil. Further laboratory studies suggested by such a review and analyses of selected, used PCB-containing industrial fluids might help to answer the above questions.

Considering the Cl-DBFs as the toxicologically most significant class of chemical impurities in the PCB mixtures, the questions may be asked: what portion of the observed effects from animal and human exposure to PCB mixtures can be attributed to the Cl-DBFs? It is doubtful this question can be answered in any quantitatively explicit way. There are analytical problems in identifying and measuring accurately the amounts of chlorinated dibenzofurans in various PCB mixtures. Even though only a relative few of the 135 possible chlorinated dibenzofurans may be identified in PCB mixtures, the toxicity of these compounds can be expected to vary with both number and ring position of chlorine atoms in the Cl-DBF molecule. Toxicity data on specific, high-purity Cl-DBFs is very limited, because the compounds themselves are not readily available. In addition to these essentially chemical difficulties, toxicological judgments would be difficult to make. To begin to evaluate the toxicological "burden" to be placed on Cl-DBF contaminants in commercial PCB mixtures, a practical approach would be to use analytical procedures that can recover and quantitate specific Cl-DBF compounds known to be highly toxic. For example, 2,3,7,8-tetrachlorodibenzofuran, already reported by Bowes (*94*) as present in PCBs, can serve as a measure of potential hazard until further toxicity data for Cl-DBFs become available.

To date, there have been no published, positive findings of Cl-DBFs in environmental samples or in foods. Analytical procedures to detect Cl-DBFs in such samples should be developed, tested and applied to appropriate samples. If chlorinated dibenzo-*p*-dioxin residue analytical findings can be taken as an indicator, procedures suitable for parts per trillion detection of Cl-DBFs will be required and quantitation will be difficult.

No clear evidence for the presence of chlorinated dibenzo-*p*-dioxins in commercial PCB mixtures has been reported. A recent publication suggesting the presence of such contaminants in some PCBs and in a synthesized chlorinated biphenyl (*29*) almost certainly misinterpreted the significance of the experimental finding related to the dioxins.

## Chemistry of Brominated Biphenyls

### Comparison with Chlorinated Biphenyls

Unlike PCBs, the chemistry and stability of PBBs have not been well studied and documented in the literature. It is difficult to assess the stability and the extent of possible chemical conversion of PBBs in the environment. PBBs can be compared chemically to the PCBs. Since both bromine and chlorine are halogens, PBB and PCB chemistry should be similar in some respects. Bromine, however, is a better leaving group in chemical reactions than chlorine. The described laboratory experiments concerning PBB alkaline hydrolysis and photolysis show that bromine is more labile than chlorine under comparable reaction parameters. PBBs may, therefore, be less stable in the environment if PBBs were under the same physical reaction parameters in the environment as PCBs. The physical state and other physical reaction parameters of PBB and PCB residues in the environment can determine reactivity. Liquids and vapors often react more readily than solids. Aroclors are liquids, though some Aroclors are extremely viscous at room temperature. Some PCBs, especially the lower chlorinated components, are volatile. FireMaster BP-6, however, is a solid and has an extremely low vapor pressure. The production, distribution, and usage of PBBs has not been as wide spread as PCBs. PBBs, unlike PCBs, may not be physically located in a position for chemical reaction. FireMaster BP-6 has been extensively used, for example, in thermoplastics, such as typewriter and business machine housings (*105*). FireMaster BP-6 has little tendency to migrate from the thermoplastic into which it is incorporated. The major portion of the products into which FireMaster BP-6 is incorporated is assumed to be buried eventually in refuse dumps. PCBs, on the other hand, are often used in transformers and capacitors and can be exposed to high temperatures which can accelerate possible chemical reactions. PCB residues are found in many diverse locations throughout most of the world. Many of these locations may offer conducive settings for chemical and metabolic conversions. PCB residues

in a body of water may react photolytically, whereas PBB residues buried in a refuse dump would not be in a position to absorb light.

## Nonmetabolic Alteration of Brominated Biphenyls

***Oxidation and Hydrolysis.*** The stability of PBBs to oxidation has not been studied and documented in the literature. FireMaster BP-6 is unstable to alkaline hydrolysis. After refluxing FireMaster BP-6 with 2% KOH in ethanol, GLC chromatograms show erratic degradation of hexabromobiphenyl (*106*), the major component of FireMaster BP-6. The possible rate of PBB hydrolysis in the environment under milder conditions is not known.

***Photochemistry.*** Recent analysis of FireMaster BP-6 by GC-MS by using an OV-101 column demonstrated 12 peaks whose mass spectrum corresponds to two pentabromo-, four hexabromo-, four heptabromo-, and two octabromo compounds. However, about 50% of the material is 1 hexabromo isomer, with the next most abundant component being the heptabromo isomer at about 20–25%. Nuclear magnetic resonance and chemical studies have identified the principal component of FireMaster BP-6 as 2,2′,4,4′,5,5′-hexabromobiphenyl (*107, 108*). The photolytic rates of reactivity of FireMaster BP-6 and 2,2′,4,4′,5,5′-hexachlorobiphenyl in methanol solutions irradiated with 3000 Å light were compared (*109*). The rate of reaction of FireMaster BP-6 was determined presumably by the decrease in the GLC peak of hexabromobiphenyl. Hexabromobiphenyl was found to be seven times as reactive as the corresponding PCB. Hexabromobiphenyl was found to undergo photolytic reductive debromination in methanol yielding penta- and tetrabromobiphenyls and also $> 1\%$ methoxylated product. Dimethoxy tetrabromobiphenyl was identified as a product by mass spectrometry. The highly efficient photoreactivity of PBB may be due to enhanced intersystem crossing to a triplet state due to vibronic coupling with the bromines, steric interference due to the bromines, and the relatively low carbon-bromine bond energy. The aromatic carbon-bromine bond energy is 71 kcal/mole, compared to the aromatic carbon-chlorine bond energy of 86 kcal/mole (*105*). As with PCBs (*18*), presumably *ortho* halogens preferentially cleave in PBBs upon photoexcitation.

The possible rates and extent of photolytic reactivity of PBBs in the environment are not known. The photochemistry of PBBs has not been studied in the vapor or solid states. Since FireMaster BP-6 has an extremely low vapor pressure, vapor-state photochemistry in the environment would be extremely limited. The extent of thin film PBB photochemistry in the environment may also be limited. PBB contamination and resulting photochemistry in river waters, except in the vicinity of PBB production facilities, may be limited. PBBs incorporated into thermoplastics, which are presumably buried eventually in a refuse dump, are not likely to absorb much light for photolytic reaction.

## Determination of Residues

Polybrominated biphenyl (PBB) residues are analyzed for in foods by methods quite similar to those used for organochlorine pesticides and polychlorinated biphenyls (PCBs) (*48, 106, 110*). Recoveries of PBB added to samples *in vivo* through these procedures is over 80%. A method to improve extraction efficiency of PBB residue from dry, high fat animal feeds has been reported (*111*).

The major difference from methods for organochlorine pesticides is in the gas chromatographic determination which is done at higher temperatures or on columns with low liquid phase loads because of the lower volatility of PBBs. Methylsilicones or Silar-10C, the liquid phases most frequently used, separate the technical PBB, FireMaster BP-6, into three to nine peaks, depending on the chromatographic conditions selected. PCBs and organochlorine pesticides which would be recovered through extraction and cleanup procedures together with PBBs have earlier retention times under these conditions and are separated from the major PBB constituent, hexabromobiphenyl. The retention times of hexabromobiphenyl and decachlorobiphenyl are not greatly different on methylsilicone liquid phases but are widely separated on Silar-10C. Elution of the Florisil column used for cleanup of extracts with petroleum ether, rather than a mixture of petroleum ether and ethyl ether, separates PBBs from most organochlorine pesticides prior to gas chromatography and improves cleanup for the PBB determination.

PBB residues are detected and quantitated with the electron capture gas chromatography detector. Quantitation is based on comparison of the size of the hexabromobiphenyl peak in the residue to the size of the hexabromobiphenyl peak in a known weight of the reference material, FireMaster BP-6. FireMaster BP-6 is a technical mixture containing 60–70% hexabromobiphenyls (*108*). The accuracy of residue quantitation is affected by any change in the composition of the residue from that of the reference technical PBB mixture. The identity of PBB residues can be confirmed by thin layer chromatography, photochemical alteration, halogen-specific

gas chromatographic detection, and by different retention times on Silar-10C and methylsilicone gas-chromatographic columns (*106, 112–114*). Mass spectrometry has also been used to confirm the identity of the PBB residue (*115*).

The limit of quantitation for PBB residues, including capability for residue identity confirmation, is about 0.05 ppm in fats and 0.01 ppm in nonfats (*106*). Formalized interlaboratory studies have not been reported for analytical methods for determination of PBB residues.

## Possibility of Brominated Dibenzofurans in Commercial PBB Mixtures

There has been no report, so far, of the finding of brominated dibenzofuran (Br-DBF) compounds in commercial PBB mixtures. Examination of FireMaster BP-6 for possible contamination by Br-DBF is in progress in several laboratories.

Thanks are due to Thomas E. Kopp, Office of Toxic Substances, EPA, for providing several background reports related to the chemistry of PCBs and PBBs.

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